Membrane Dynamics II

1221-Pos Board B172
PIE-FCCS Study of the Effects of Polycationic Macromolecules on Phosphatidylserine and Phosphatidylinositol Phosphate Lipid Mobility
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The interaction between proteins and anionic lipids in the plasma membrane is a common motif in cell communication at the plasma membrane. Such interactions can gate membrane protein function and have also been proposed to sequester membrane lipids during quiescent phases of signaling. To date, however, the molecular structure and dynamics of the lipid-protein interface is poorly understood. To isolate these interactions in a biophysical assay, we have investigated the behavior of a polycationic polymer, quaternized polyvinylpyridine (QPVP), on supported lipid bilayers doped with tail-labeled phosphatidylserine (PS) or phosphatidylinositol phosphate (PIP) lipids. To measure the mobility and association of the lipid and adsorbed polymer, we use a time-resolved fluorescence technique, pulsed interleaved excitation fluorescence cross-correlation spectroscopy (PIE-FCCS). PIE-FCCS is a dual-color fluorescence spectroscopy that translates fluctuations in fluorescence signal into a measurement of diffusion and co-localization. With PIE-FCCS we have investigated the polymer adsorption-dependent translational mobility of the lipids and systematically studied the influence of lipid head-group charge and solvent ionic strength. Our results indicate that alteration of anionic lipid lateral mobility is dependent on the net charge of the lipid head group and is significantly screened by the ionic strength of the solution. At physiological salt concentration we observe that the lipid lateral mobility is nearly unaffected by mobile, adsorbed polymers and that there is no evidence of stable lipid-polymer complexes.

1222-Pos Board B173
The Effects of Ca^{2+} on the Dynamics of PIP2 Containing Lipid Bilayers
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Changes of intercellular Ca^{2+} concentrations are one of the most ubiquitous signaling events that accompany or precede large scale cellular responses. We are in particular interested in the direct modulation of phosphatidylinositol 4,5-bisphosphate (PIP2) interactions by such changes in Ca^{2+} levels and the associated changes in PIP2 organization in biomembranes. To that end a series of experiments were conducted on 1,2d-sn-glycero-3-phosphocholine (DOPC) bilayers supported by a cushion of poly (l-lactic acid) (PLLA) containing also physiological quantities of PIP2. Fluorescence correlation spectroscopy was used to study the response of PIP2 to changes in the concentration of Ca^{2+} ions that cover the intercellular calcium levels during transitions from the resting to an excited cell state (0-1000nM). After an initial increase in motility up to about 100nM Ca^{2+}, PIP2 diffusivity is found to decrease with increasing calcium concentration. This behavior can be explained by the initial break-up of pre-formed PIP2-DOPC clusters and the subsequent Ca^{2+} induced aggregation of PIP2.

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Lipid Dynamics of Cardiolipin/DMPC and Cardiolipin/DOPC in Nanodiscs
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Cardiolipin is a negatively charged phospholipid that comprises roughly 20% of the lipids in the inner mitochondrial membrane. The dynamics of this membrane can influence interactions between cytochrome c and cardiolipin, which have been shown to initiate the intrinsic pathway of apoptosis. This investigation studies how the dynamics of a model membrane system of varying composition will behave in the presence and absence of cardiolipin. Nanodiscs are composed of varying concentrations of 18:1 cardiolipin and DOPC or DMPC, doped with either NBD-PE or TMA-DPH. (18:1 cardiolipin: 1′,3′-bis[1,2-dioleoyl-sn-glycero-3-phospho]-sn-glycerol DMPC: 1,2-dimyristoyl-sn-glycero-3-phosphocholine DOPC: (Δ9-Cis) PC 1,2-dioleoyl-sn-glycero-3-phosphocholine NBD-PE: 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl) TMA-DPH: trimethylammonium diphenylhexatriene). Time-Resolved Emission Spectra (TRES) of NBD-PE doped nanodiscs are used to report on head group relaxation and Time-Resolved Fluorescence Anisotropy of TMA-DPH doped nanodiscs are used to report on acyl chain motions. These methods are combined to provide insight into how cardiolipin affects lipid membrane dynamics.

1224-Pos Board B175
The Cytotoxic Bile Acid DCA Modulates Apoptotic Signalling through Alteration of Mitochondrial Membrane Properties
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Deoxycholic acid (DCA) and other hydrophobic bile acids induce apoptosis at submicellar concentrations, while bile acids such as ursodeoxycholic acid (UDCA) and its taurine conjugate (TUDCA) display cytoprotective properties. The mechanisms that trigger these opposite signalling effects are still unclear. Recent studies have confirmed that cytotoxic bile acids decrease membrane order in membrane model systems and in purified plasma membrane vesicles, suggesting that cytotoxicity could be initiated through modulation of plasma membrane structure. Using confocal microscopy and two-photon fluorescence microscopy of Laurdan in hepatocytes and established cell lines, we have shown that upon uptake, bile acid analogues accumulate in intracellular membranes and display remarkable low plasmalemmal levels. Incubation of hepatocytes with both classes of bile acids resulted in a dramatic decrease in intracellular membrane order, as a result of bile acid accumulation during uptake. Bile acids also accumulated in mitochondria, but only DCA induced significant changes in the membrane order of isolated mitochondria. Importantly, the DCA induced changes in membrane fluidity preceded an increase in mitochondrial permeability, which is not detected for both UDCA and TUDCA. Our results are consistent with the presence of cellular compensatory mechanisms, which work against the moderate loading of bile acids in the plasma membrane, but that are unable to balance the increase in membrane fluidity induced by cytotoxic bile acids in mitochondrial membranes. Our findings suggest that apoptosis induced by cytotoxic bile acids is the result of changes in mitochondrial membrane structure after incorporation of bile acids.
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Mechanics of Extracellular Vesicles from Red Blood Cells
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Extracellular vesicles (EVs) are endogenous particles involved in cell to cell communication. EVs transport proteins and RNA, appear to play a role in disease and are potentially useful as a drug delivery system. The stiffness of these vesicles might be important for their functioning because it is believed that it may play a role in e.g. endocytosis by cells. We look into the mechanics of extracellular vesicles excreted by red blood cells (RBC EVs) using atomic force microscopy (AFM), and compare them with liposomes with a similar lipid composition. AFM imaging shows that RBC EVs (~ 125 nm in diameter) stay in a spherical shape upon interaction with a poly-L-lysine coated surface, whereas similar sized liposomes adopt a hemi-spherical shape. Indentations of both liposomes and RBC EVs reveal thin shell behavior, characterized by a linear rise in force and subsequent buckling. Surprisingly, the liposomes seem stiffer. However, when we correct for (i) liposome spreading that leads to an internal osmotic pressure which stiffens the response as well as for (ii) the different geometry of the vesicles, we observe that RBC EVs are indeed significantly stiffer. This result show that EVs indeed have a higher stiffness than can be expected solely from their lipid composition and dimensions.

1226-Pos Board B177
Modeling of Vesiculation in Healthy and Defective Human Erythrocyte Membrane
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We apply a two-component coarse-grained molecular dynamics (CGMD) red blood cell (RBC) membrane model which includes an explicit representation of the spectrin network, the lipid bilayer, and the band-3 proteins, to simulate